ESTONIAN UNIVERSITY OF LIFE SCIENCES

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Estrotect © as an indicator of fertile heat compared to visual detection in dairy heifers

Estrotect©'i kasutamise ja tavapärase visuaalse innaavastamise järel teostatud seemendustulemuste võrdlusanalüüs piimakarja mullikatel

Final thesis in Veterinary Medicine

Curriculum in Veterinary Medicine

Supervisors:

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ABSTRACT

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Heat detection and the precise timing of animals in true estrus has become one of the most important challenges in modern-day dairy farming. Additionally, replacement animals are more often artificially inseminated. A well-managed replacement herd breeding program is crucial for a farm to achieve the most economical profit.

In this study, we compared Estrotect heat detection devices with ordinary visual heat detection (two times per day). The aim was to compare the results of using heat detection aids with the labor-concerning practice of visual heat detection. A total of 142 Holstein and 30 Estonian Red breed heifers were selected as a group inseminated according to Estrotect devices; for the second group, heat was detected visually in 92 Holstein and 14 Estonian Red breed heifers. All animals selected to this study were inseminated for the first time. When inseminated according to Estrotect device, 66.9% of Holstein heifers and 36.7% of Estonian Red heifers became pregnant. When heat was detected visually, 71.7% of Holstein heifers and 71.4% of Estonian Red heifers became pregnant. According to this study, Estrotect can be a useful aid to improve the fertility results of replacement herds in farms with problems related to visual heat detection management.

Keywords: Heifer, Fertility, Pregnancy rate, Visual heat detection, Estrotect

LÜHIKOKKUVÕTE

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Kreutzwaldi 1, 51014, Tartu</td><td colspan="2">Lõputöö lühikokkuvõte</td></tr>
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<tr><td colspan="4">Pealkiri: Estrotect©’i kasutamise ja tavapärase visuaalse innaavastamise järel teostatud seemendustulemuste võrdlusanalüüs piimakarja mullikatel</td></tr>
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Juhendaja(d): Kalle Kask, Tanel Kaart
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Töös kasutati looma sabajuurele kleebitavat visuaalset inna- avastamise abivahendit Estrotect (Rockway, Inc., Tennessee, United States) ja analüüsiti mullikate tiinestumist, keda seemendati ainult selle abivahendi alusel avastatud innast. Võrrelduna mullikate tiinestumisega, keda seemendati tavapärase hommikusel ja õhtusel inna-avastamisel leitud innatunnuste järgi. Estrotect’i kasutamine põhineb looma sabajuurele kinnitatud kleebise värvimuutusel. Kui loomal on püsiind, siis ta talub teiste indlevate loomade hüppamist tema laudjale ja nende korduvate hüppamiste tagajärjel toimub sabajuurele kinnitatud Estrotect’i kleebise värvuse muutus. Mida intensiivsem on värvi muutus, seda tõenäolisem on see, et loomal on püsiind ja me saame teostada selle alusel seemenduse. Töös kasutatud mullikad jagati kahte katserühma. Esimesse katserühma kellele paigaldati Estrotect’i kleebis valiti 142 Holsteini tõugu ja 30 Eesti punast tõugu mullikat. Teise katserühma keda seemendati tavapärase, hommikusel ja õhtusel inna avastamisel leitud innatunnuste järgi, valiti 92 Holsteini tõugu ja 14 Eesti punast tõugu mullikat. Inna avastamine teostati hommikul kella 07:00 ka kella 09:00 vahel ja õhtul kella 17:00 ja 19:00 vahel. Samadel kellaaegadel toimus ka kontroll Estrotecth’i kleebise värvimuutusele. Innatunnused, mida jälgiti selle rühma loomadel, kellel toimus tavapärane rutiine inna avastamine olid järgmised: teise looma laudjale hüppamise talumine, aktiivsusprofiil, häälitsemine, pea toetamine teise looma laudjale, limavool häbemest. Mõlema katserühma mullikatel olid teostatavad seemendused esmakordsed. Loomade vanus jäi vahemikku 12.5 kuni 15 kuud. Tiinestumise määr esimesest seemendusest rühmas kus kasutati Estrotect’i oli madalam nii Holsteini, kui ka Eesti punast tõugu mullikatel. Holsteini mullikatel Estrotect'i rühmas tiinestus 66.9±4.0% ja rühmas, kus seemendused teostati visuaalsel jälgimisel saadud innatunnustele põhinedes oli tiinestumine 71.7±4.7% (p=0.436). Eesti punast tõugu mullikatel oli tiinestumise määr vastavalt 36.7±8.8% Estrotect'i rühmas ja 71.4±12.1% (p=0.037) rühmas, kus seemendused teostati visuaalsel jälgimisel saadud innatunnustele põhinedes. Kokkuvõttes ei leitud statistilist erinevust inna avastamise metoodika vahel (p=0.129). Statistiline analüüs näitas samuti seda, et tõug ei mõjuta inna avastamise metoodikat (p=0.105). Samal ajal oli tiinestumise määr katses kasutatud Eesti punast tõugu mullikate statisitiliselt madalam kui kasutatud Holsteini mullikatel (p=0.012). Teostatud uuring näitas, et farmid, kes pole rahul oma noorkarja tiinestumis näitajatega ja mille võimalik põhjus võib olla probleemne inna avastamine, võiksid kasutada Estrotect'i kleebiseid inna avastamise parandamiseks. Loomade grupeerimine ja kleebiste paigaldamine on küll mõnevõrra aeganõudev, aga kokkuvõttes on võimalik hoida kokku aega, mis kulub rutiinsele kahekordsele päevasele inna avastamisele.

Märksõnad: Mullikad, tiinestumise määr, visuaalne inna avastamine, Estrotect.

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LIST OF ABBREVIATIONS

VHD Visual Heat Detection

AI Artificial Insemination

et al. Citated source has three or more authors. See list of references

FSH Follicle Stimulating Hormone

LH Luteinizing Hormone

CL *Corpora Lutea,* Corpus Luteum

BW Birth Weight

EPD Expected Progeny Difference

1. INTRODUCTION

The goal of reproductive management is to have cows and heifers become pregnant at a biologically optimal time and at an economically profitable interval after calving (Sakaguchi, 2011). Efficient and profitable reproductive performance of a dairy herd requires routine but conscientious heat (estrus) detection and proper timing of artificial insemination (AI; O'Connor, 1993). Any factor that even slightly improves reproductive performance can have a large effect on the efficiency of food animal production (Senger, 2012). One of the most important factors in herd fertility is estrus detection (Van Eerdenburg, 1996). For accurate and efficient detection, the primary and secondary signs of estrus are essential (O'Connor, 1993). Failure to detect estrus is a major factor contributing to low fertility (O'Connor, 1993).

Good management practices in semen storing and handling help to minimize the errors in the thawing and maximize the results of performed insemination (Saacke, 1974). In most domestic species, the period of estrus is less than 24 hours. In other words, copulation must take place within a period that is close to ovulation (Senger, 2012). A study on beef cattle breeding by Patterson et al. (2013) concluded that traits of major economic importance in beef cattle can be improved most rapidly through selection of genetically superior sires and widespread use of AI. According to many studies (Mourits et al., 1999; Abeni et al., 2000; Gabler and Heinrichs, 2003; Shamay et al., 2005; Stevenson et al., 2008), the optimal age for first calving is younger than 24 months. Breeding dairy heifers at this younger age reduces costs by shortening their nonproductive period.

In the case of problems in heat detection or achieving the correct insemination time or first insemination age, or other management- and labor-affected problems, it is possible to use different estrus detection aids. Pressure-sensitive devices activate according to the mounting behavior of other animals. Additionally, collar devices measure the activity of the animals, and mechanical coloring techniques use paints and chalks that rub off when the animals mount each other. In this study, we compared pressure-sensitive devices to visually noted estrus signs.

2. ESTROUS CYCLE OF THE CATTLE

2.1. Estrous

Polyestrous female mammals, such as cattle, swine, and rodents, are characterized as having a uniform distribution of estrous cycles throughout the entire year. Polyestrous females can come pregnant throughout the year without regard to season (Senger, 2012). The estrous cycle can be divided into two distinct phases that are named after the dominant structure present on the ovary during each phase of the cycle: the follicular phase and the luteal phase (Senger, 2012).

2.2. Follicular phase

The follicular phase is the period from the regression of corpora lutea to ovulation (Senger, 2012). In general, the follicular phase is relatively short, encompassing approximately 20% of the estrous cycle (Senger, 2012).

2.2.1. Proestrus

Proestrus last from 2 to 5 days, depending on the species, and is characterized by a major endocrine transition from a period of progesterone dominance to a period of estradiol dominance (Senger, 2012). Proestrus is the period when progesterone declines with regression of the corpus luteum, estrogen increases, and secondary signs of estrus begin to occur (O'Connor, 1993). The pituitary gonadotropins, follicle stimulating hormone and luteinizing hormone, are the primary hormones responsible for this transition (Senger, 2012).

2.2.2. Estrus

Estrus is the period of sexual receptivity and is commonly referred to as heat (Senger, 2012). Estrus is characterized by standing behavior (true heat) (O'Connor, 1993). In mammals, estrus is a behavioral symptom and strategy to ensure that the female is mated close to the time of ovulation. Estrus is an external and visible sign of ovulation, an internal and invisible event (Roelofs et al., 2010). Estrus lasts 12–24 h, and its predominant hormone is estradiol, which is responsible for the heat signs the animals express by acting on the central nervous system. The expression of heat is due to the elevated estrogen levels in the blood when progesterone is very low (O'Connor, 1993). Estradiol not only induces profound behavioral alterations but causes major physiologic changes in the reproductive tract (Senger, 2012).

2.3. Luteal phase

The luteal phase is the period from ovulation until corpora lutea regression (Senger, 2012). The luteal phase is much longer than the follicular phase and, in most mammals, occupies approximately 80% of the estrous cycle (Senger, 2012)

2.3.1. Metestrus

Metestrus is the period between ovulation and the formation of functional corpora lutea. During early metestrus, both estradiol and progesterone are relatively low (Senger, 2012). The newly ovulated follicle undergoes cellular and structural remodeling, resulting in the formation of an intraovarian endocrine gland called the corpus luteum (Senger, 2012). Loss of follicular size leads to a decrease in corpus luteum cells that form from granulosa cells after ovulation, and this is thought to be the reason for smaller corpus luteum formation, of less secreted progesterone after ovulation, and a lower final pregnancy rate after timed artificial insemination (Perry et al. 2007).

Progesterone secretion begins in metestrus and is detectable soon after ovulation. However, 2–5 days are usually required after ovulation before the newly formed corpora lutea produce significant quantities of progesterone (Senger, 2012).

2.3.2. Diestrus

Diestrus usually lasts approximately 10–14 days in most large mammals (Senger, 2012). Diestrus is the longest stage of the estrous cycle and is the period when the corpus luteum is fully functional and progesterone secretion is high (Senger, 2012). The duration of diestrus is directly related to the length of time that the corpus luteum remains functional (i.e., secretes progesterone) (Senger, 2012). It ends when the corpus luteum is destroyed (luteolysis). Females in diestrus do not display estrous behavior (Senger, 2012).

If the ovulated oocyte is fertilized and pregnancy is established, the corpus luteum will not regress during the next cycle. The progesterone production will be sustained to maintain the pregnancy (Senger, 2012).

3. EXPRESSION OF ESTRUS

One of the most important factors in herd fertility is estrus detection. For good estrus detection, two major requirements need to be met: (1) a skilled observer must spend sufficient time on observations of the herd and (2) cows have to show overt signs of estrus during those observations (Van Eerdenburg, 1996). It is also essential to understand the primary and secondary signs of heat in order to achieve accurate and efficient heat detection (O'Connor, 1993).

When a female enters estrus, she does so gradually and is not sexually receptive at first. She may display behavioral characteristics that are indicative of her approaching sexual receptivity. These include increased locomotion, phonation (vocal expression), nervousness, and attempts to mount other animals (Senger, 2012).

The expression of estrus can be influenced by many factors. Heritability, number of days postpartum, lactation number, milk production, and health are known to influence estrus expression. Additionally, environmental factors such as nutrition, season, housing, and herd size play a role in estrus expression (Roelofs et al., 2010). Because cows do not show their estrous behavior at a specific time of the day, and estrous periods are sometimes very short, the cows can be observed best after milking and feeding in the morning, early afternoon, and evening. Observation periods should last more than 20 minutes to be effective (Van Eerdenburg, 1996).

3.1. Primary signs

3.1.1. Standing heat

The willingness of the female to accept the male for mating is referred to as standing heat (Senger, 2012). It is during the time of estrus that the female displays a characteristic mating posture known as lordosis, named for a characteristic arching of the back in preparation for mating (Senger, 2012).

Standing heat is the most sexually intensive period of the estrus cycle (O'Connor, 1993). It is considered the most discriminative sign of estrus (Holtz et al., 1993). Standing behavior (lordosis) is easily observed and is used as a diagnostic tool to identify the appropriate time to inseminate the female artificially or expose her to the breeding male (Senger, 2012).

During this period, cows stand to be mounted by other cows or move forward slightly with the weight of the mounting cow. Cows that move quickly away from the mounting attempt are not in true estrus.

For standing behavior to be expressed, cattle must be allowed to interact (O'Connor, 1993). A cow standing to be mounted is the most specific and accurate sign of estrus (Diskin et al., 2000), but 37–54% of detected ovulations are not accompanied by standing estrus in Holstein cows (Van Eerdenburg et al., 1996; Lyimo et al., 2000; Roelofs et al., 2005; Sakaguchi, 2011).

The average duration of standing heat is 15–18 hours, but heat duration may vary from 8 to 30 hours among cows. An estrous cow usually stands to be mounted 20–55 times during her estrus period. Each mount lasts from 3 to 7 seconds (O'Connor, 1993). In the Holstein breed, the duration of estrus was approximately 18–20 hours in the 1980s, but since the early 2000, it has shortened to only 4–8 hours between the first and the last standing mount, or 11–14 hours if all signs of estrus are taken into account (Van Vliet et al., 1996; Kerbrat et al., 2004; Roelofs, 2005; Chanvallon et al., 2014).

3.2. Secondary signs

3.2.1. Mucus discharge

Discharges seen at the vulva can be sometimes confusing. Dairymen cannot always be sure if the discharge indicates trouble or if it is a natural secretion. Generally, the secretions that appear at the vulva accurately mirror the current condition of the reproductive tract (Woelffer, 1973).

Mucus discharge is an indirect result of elevated estrogen levels. Mucus is produced in the cervix and accumulates with other fluids in the vagina before, during, and shortly after estrus. Long, clear strands of viscous, elastic-like mucus generally hang from the vulva. However, sometimes, the mucus does not appear externally until the cow is palpated during insemination and the mucus is expelled (Figure 1). Additionally, mucus may be smeared on the tail, surfaces, flanks, or perineal region (O'Connor, 1993).

The discharge seen most often is crystal clear, resembling the white of a raw egg. This is the normal discharge associated with heat. In some individuals, it is plentiful (Figure 2). In others, it is less conspicuous. However, in some cows, it might not be noticeable at all (Woelffer, 1973).

Although very often used by farmers, mucous vaginal discharge is very unreliable as an indicator for estrus. When a long string (>50 cm) of clear, viscous mucus hangs from the vagina, the cow can be considered to express estrus (Van Eerdenburg, 1996).

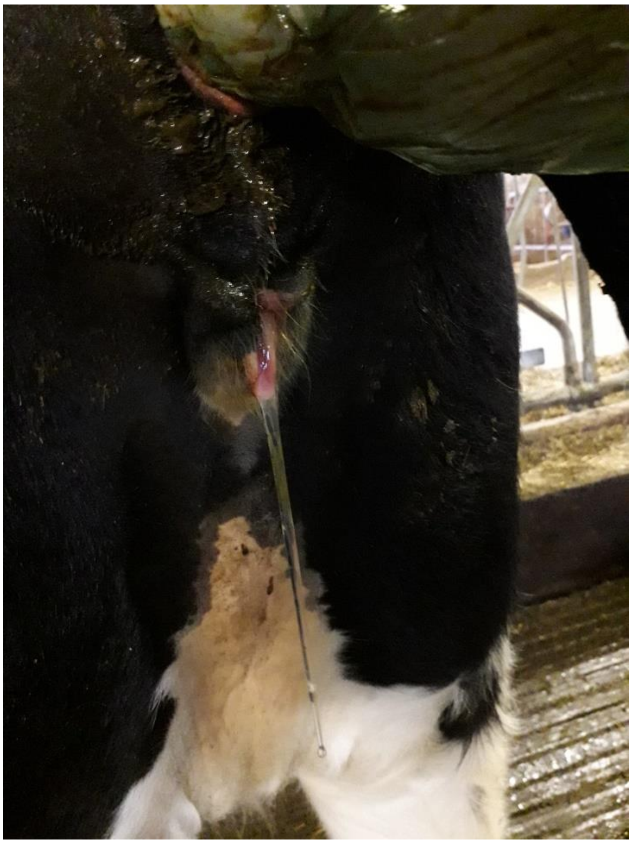

Figure 1. Mucus expelled when palpating the uterus. Photograph: P. Lehtinen, 2019.

Figure 2. Plentiful mucus discharge in a resting cow. Photograph: P. Lehtinen, 2019.

A generous flow of material indicates that the secreting glands are functioning normally. If the clear discharge is tinged with red or is markedly red (Figures 3 and 4), it means that the cow has gone out of heat, usually a couple of days earlier (Woelffer, 1973). Some cows and most heifers have a bloody mucus discharge 1–3 days after estrus, but the onset of this symptom, called metestrus bleeding, is quite variable. High estrogen levels during estrus cause blood to leak from the vessels near the surface of the uterus. This discharge indicates that the cow was in heat and does not mean that she failed to conceive (O'Connor, 1993).

Figure 3. Metestrus bleeding in a cow. Photograph: P. Lehtinen, 2018.

Figure 4. Metestrus bleeding in a heifer. Photograph: P. Lehtinen, 2019.

3.2.2. Chin resting and back rubbing

Prior to mounting, cows often rest or rub their chin on the rump or back of the cow to be mounted (Figure 5). This application of pressure may be considered a test for the receptivity to being mounted. Both cows should be observed closely for mounting and standing behavior (O'Connor, 1993).

Resting with the chin on another cow occurs four times as often in estrus as in diestrus and is therefore a good indicator of estrus (Van Eerdenburg, 1996).

Figure 5. Chin resting prior to mounting. Photograph: P. Lehtinen, 2019.

3.2.3. Mounting

Cattle that exhibit this behavior may be in heat or approaching heat. Although performed much less frequently by cows in midcycle, mounting cannot be used as a true primary sign of heat (Figure 6). Cows exhibiting such behavior should be watched closely for standing behavior (O'Connor, 1993). However, other study has found that the onset of mounting is the best predictor for time of ovulation (occurring 28.7 ± 5.3 h before ovulation), and it was displayed in 90% of the estrous periods (Roelofs et al., 2005).

Mounting, or attempting to mount, other cows is one of the most accurate external signs of estrus (Holtz et al., 1993). However, mounting also occurs in diestrus (Van Eerdenburg, 1996). Esslemont and Bryant (Esslemont and Bryat, 1976) considered a cow as being in estrus when she mounted another cow at least six times a day. Therefore, other signs of estrus, in addition to mounting (e.g., chin resting), are needed to be confident that the cow is in estrus (Van Eerdenburg, 1996). In all cases, estrus is better expressed when several cows are simultaneously in heat (Diskin et al., 2000; Roelofs et al., 2005).

Figure 6. Standing and mounting activity. Photograph: P. Lehtinen, 2019.

4. ESTROUS DETECTION

Estrus in dairy cattle varies in duration and intensity, highlighting the need for accurate and continuous monitoring to determine optimal breeding time (Mayo et al., 2019). Efficient and accurate detection of estrus is vital to good reproductive performance in a dairy herd where AI is used (Kinsel et al., 1998) and, in turn, is essential to maintain profitability (Van Vliet et al., 1996). Problems with estrus detection can lead to major financial implications for farmers and an increase in the number of cows culled for infertility (Walker et al., 1996). In recent decades, the fertility of dairy cows has declined, with a contributory factor being a decline in estrus expression (Mee, 2004).

Traditionally, estrual cows were identified by visual observation. As herd size increases, visual observation of individual cows is not practical within the available time of the herd manager, resulting in unobserved estrus and remarkable economic losses (Reith et al., 2018). Detection efficiency is often below 50% in dairy herds (Van Vliet et al., 1996). Although poor reproductive performance causes the highest culling rate, few cows are described to be infertile. Approximately 90% of the factors for low detection rates can be attributed to management and 10% to the cow (Diskin et al., 2000). Due to the high variability in duration and intensity of the expressed estrous signs among individual cows and the great influence of a number of various factors, detection of estrual cows is still a major problem (Reith et al., 2018).

4.1. Visual detection

For good estrus detection, two major requirements need to be met: (1) a skilled observer must spend sufficient time on observations of the herd and (2) cows have to show overt signs of estrus during those observations. (Van Eerdenburgh et al., 1996). The ability to detect a cow in estrus primarily depends on the experience of the farmer and the time invested in estrus detection (Roelofs et al., 2010). The most widely used method of estrus detection is visual observation, and high detection rates can be achieved with between two (Xu et al., 1998) and five (Mee, 2004) 20–30 min observation sessions per day. Because cows do not show their estrous behavior at a specific time of the day, and estrous periods are sometimes very short, the cows can be observed best after milking and feeding in the morning, early afternoon, and evening (around 2000 h). Observation periods should last more than 20 minutes to be effective (Van Eerdenburg et al., 1996). Estrus detection efficiency by visual observation of standing heat, as mentioned in different studies, vary widely from 90% to less than 50%.

Therefore, it is clear that signs other than standing heat are important to detect cows in estrus (Roelofs et al., 2010).

4.2. Detection aids

Estrus detection aids can help to improve estrus detection (Roelofs et al. 2010). The need for this critical but time-consuming task can be avoided altogether by using the hormonal induction of estrus, a practice that is now widespread, particularly in the United States (Caraviello et al., 2006). These programs aim to induce synchronous ovulation, thus allowing fixed-time insemination without the need for heat detection. In Europe, the use of hormonal programs to synchronize estrus is not so widespread due to the cost of treatment and the reluctance of European consumers to accept products from animals treated with hormones or antibiotics (Chanvallon et al., 2014).

Another aid in estrus detection is mount detection devices. These devices are attached to the sacrum of the cow and indicate whether a cow has been mounted or not (Figure 7). A nonelectronic variant of mount detection is the use of tail paint. Detection rates vary among studies from under 50% (Peralta et al., 2005), to as high as 80% (Shipka, 2000), and over 85% (Cavalieri et al., 2003). The efficiency of the devices is affected by the threshold that is set for identifying estrus and the way the device is attached to the animal. However, such devices do not work properly when brushes are present in the stable. Additionally, cow and environmental factors affect estrus detection efficiency (Roelofs et al., 2010).

Figure 7. Activated Estrotect, a mount detection device. Photograph: P. Lehtinen, 2019.

Increased physical activity is a secondary sign of estrus in cattle, and pedometry systems that detect changes in the number of steps per unit time have been available for many years (Fricke et al., 2014). US dairy farmers have shown a clear trend toward the adoption of new technologies such as activity monitoring systems for detecting estrus in dairy cows. At present, activity monitoring systems can accurately detect approximately 70% of cows in estrus (Fricke et al., 2014).

5. ARTIFICIAL INSEMINATION

AI provides the opportunity to breed heifers to bulls selected for low birth weight or high calving-ease expected progeny difference with high accuracy. This practice minimizes the incidence and severity of calving difficulty and decreases calf loss that results from dystocia. Finally, heifer calves that result from AI can be an excellent source of future replacements, facilitating more rapid improvement in the genetic makeup of an entire herd (Patterson et al., 2013).

5.1. Thawing of semen

Most bull semen is now frozen in either 0.25 or 0.50 ml French straws and stored in liquid nitrogen at −196°C (Diskin, 2018). The handling of semen, particularly the 0.25 ml straw, is critically important. Thawed semen needs to be protected from cold and heat shocks and inseminated within 6–8 min of thawing (Diskin, 2018). A major disadvantage with straws is their vulnerability to mishandling, particularly the 0.25 ml straws, which are most popular in Europe and Canada. (Diskin, 2018)

The 0.25 ml straws have a larger surface-to-volume ratio than the 0.5 ml straws, which makes them vulnerable to rapid temperature fluctuations. It is recommended that 3 seconds is the maximum time for moving 0.25 ml straws from one liquid nitrogen tank to another without damaging the sperm and similarly for moving from tank to thaw flask (Seidel, 2011). Rapid thawing decreases the harmful effects of water recrystallization and rehydration, thus preventing damage to sperm membrane and cytoplasm. The critical temperature zone for ice crystals to form is between −50°C and 0°C. Rapid progression though this temperature zone means that the semen switches from a glassy state to a liquid one, and ice crystals have insufficient time to form (Diskin, 2018).

Good management practices in semen storing and handling help to minimize the errors in the thawing and maximize the results of performed insemination. In the nitrogen tank, the use of clearly labeled straws and goblets helps to quickly select the correct straw and minimize the time the tank is kept open. When locating the straws, the canister should be kept below the frost line; lifting it for too long in the process should be avoided. If a semen straw cannot be located within 10 seconds, the canister should be lowered into the liquid nitrogen before continuing the search (Saacke, 1974). The straws should be handled and removed from the tank using forceps. Any excess nitrogen should be removed from the straw before placing it in the water bath at 35°C for 45 seconds (range, 30–60 seconds). Only sufficient straws that can be inseminated with 10 min should be thawed (Diskin, 2018). If multiple straws are thawed at the same time, direct straw-to-straw contact should be avoided during thawing process. The AI gun should be prewarmed and kept warm. The straw should be removed from the water bath, wiped dry, and inserted into the AI gun. Temperature changes to the straw should be avoided, and the animal should be inseminated as soon as possible.

5.2. Site of insemination

In the initial insemination studies in cattle in the 1940s, the semen was typically deposited in the vagina or inside the external os of the cervix. This was followed by studies where a hand was placed in the rectum to hold the cervix while the semen was deposited deep into the anterior cervix or uterus (Diskin, 2018). It has been erroneously assumed for years that most spermatozoa ascend toward the oviduct soon after they are deposited in the cow uterus by AI. However, recent studies have shown that a high proportion of spermatozoa deposited in the uterus of the cow or ewe are lost from the tract by retrograde transport (Senger, 2012). In most cows, over 60% of spermatozoa artificially inseminated into the uterus are lost to the exterior of the tract within 12 hours after deposition (Senger, 2012).

The results of several studies (Knight et al., 1951; Salisbury and VanDemark, 1951; Stewart and Melrose, 1952; Olds et al., 1953) have shown little differences in fertility when semen was deposited in the uterine horns, uterine body, or indeed mid-cervix. This resulted in the uterine body being seen as the accepted site of semen deposition when AI is employed with cattle (Diskin, 2018). Notably, these early studies used fresh semen and high sperm count per insemination, especially because nowadays, primarily frozen-thawed semen is used with lower sperm count. With conventional semen, 15–30 × 10^6 sperm are used (Diskin, 2018). Based on the findings of Gallahger and Senger (1989), a logical interpretation would be that the AI of spermatozoa deep into the uterus would result in reduced retrograde loss. However, when sperm are deposited deep into both uterine horns (as opposed to the uterine body), the degree of sperm recovered from the vagina (an indication of retrograde loss) is quite similar between the two sites of deposition. When sperm are deposited in the mid-cervix, a significantly higher degree of retrograde loss of spermatozoa is encountered (Senger, 2012).

5.3. Timing of insemination

The objective of an insemination is to ensure that an adequate reservoir of competent, capacitated, motile sperm is present in the caudal region of the oviductal isthmus at the time of ovulation to ensure the highest possible fertilization rate (Diskin, 2018). The exact lifespan of sperm cells has not been determined, but according to the literature and data derived from mated animals, the estimated lifespan of bull semen is approximately 30 hours. It is uncertain whether the viable lifespan of the sperm represents an intrinsic property of the sperm cell or whether it is influenced by the uterine environment (Diskin, 2018).

An interval of 6–12 hours has been estimated for sperm to move from the site of semen deposition to the caudal region of the oviductal isthmus (Hawk, 1987). When insemination takes place too early, the sperm is aged and by the time ovulation occurs, it cannot fertilize the ovum (Hawk, 1987). the highest pregnancy or conception rates have been found when insemination is performed between 5–17 hours after an increase in general activity (Maatje et Al., 1997; Roelofs, 2008). When insemination takes place too late, the egg is aged, and fertilization and formation of a viable embryo is not likely (Hunter, 1997).

The cattle ovum appears to have a short lifespan after ovulation before degenerative changes are initiated (Diskin, 2018). Estimates of the lifespan of the ovulated ovum vary from 8–12 hours (Laing, 1945; Casida, 1950) to 20–24 hours (Thibault, 1967). In a study based on heat watch technology, Dalton et al. (2001) concluded that insemination at 12 hours after onset of estrus provided a compromise in terms of maximizing fertilization and embryo survival rates. This conclusion is in agreement with data from Dransfield et al. (1998), who reported the optimal time for insemination as 4–16 hours after estrus onset. Furthemore, Dalton et al. (2001) suggested that embryo quality following late insemination may be impaired due to an aging ovum at the time of fertilization.

However, in practice, the exact time of estrous onset is rarely known, and combined with the inter-animal variation in the timing of ovulation, it is not practical to recommend an exact timing for insemination (Diskin, 2018). The early pioneering work of Trimberger and Davis (1943) and Trimberger (1948) showed that maximum conception rates could be obtained when cows are inseminated from midway to the end of standing estrus. Additionally, satisfactory results were obtained when inseminations were performed during the 6 hours following the end of standing estrus. This work lead to the well-established and recommended AM-PM rule, which still stands today (Diskin, 2018). These early studies (Trimberger et Al. 1944; 1943) were based on very frequent (4–12 times per day) estrus detection of cows, and inseminations done on standing heat, not secondary signs. These studies indicated that maximum pregnancy rates were obtained from mid-estrus until a few hours after the end of standing behavior (Roelofs et Al., 2010). On the contrary, studies with twice daily heat checks, using several thousands (44,707) of fresh or frozen semen, did not observe any differences in nonreturn rates for cows that received AI at the same AM or during the PM following AM detection (Foote, 1978).

If the pregnancy rate is unsatisfactory or heat is not routinely detected, cows should be inseminated soon after they are first detected in standing heat. If the beginning of estrus is more precisely detected using an electronic estrus detection system, it would be better to inseminate within 4–12 hours of observed estrus (Dransfield et Al., 1998).

5.4. Artificial insemination technique in bovines

AI technique requires that the spermatozoa be deposited in the reproductive tract of the female by artificial means (Senger, 2012). In general, semen is delivered using a pipette to penetrate and bypass the cervix. This type of insemination is referred to as transcervical insemination (Senger, 2012).

6. AGE AT FIRST CALVING

Holstein heifers achieve puberty at 30–40% of their expected adult body weight. The recommended body weight for insemination is between 340 and 365 kg, while the recommended age at first calving is considered to be 24 months or younger with body weight of at least 560 kg after calving (Heinrichs, 1993; Tozer and Heinrich, 2001). The productive life, milk yield, reproductive performance, and health of primiparous Holstein cows is closely related to age at first calving and weight immediately after calving (Gabler et al., 2000; Ettema and Santos, 2004). Optimum fertility and maximum yield in the first lactation were associated with an age at first calving of 24–25 months. However, heifers calving at 22 to 23 months performed best in terms of total milk yield and survival over the first 5 years, partly because good heifer fertility was associated with better fertility later (Wathes et al., 2008).

Hoffman et al. (1996) concluded that the possible advantages of reduced age at first calving, such as decreased feed costs, greater cumulative production per month of age, shorter generation interval, and lower overhead costs, must be weighed against possible disadvantages such as lower conception rates, increased dystocia, reduced milk production per lactation, diminished longevity, and the costs of increased nutrient density in the ration. Lower feed efficiency would be expected after calving from first lactation cows that are still growing at a fast pace when coming into milk.

Dairy farmers face a complex dilemma in minimizing costs associated with rearing heifers while ensuring or enhancing lifetime economic productivity. Decisions about heifer management interact with underlying biological aspects of growth, thereby influencing future profitability of the herd (Mourits et al., 1999). A basic approach to reduce costs is to shorten the nonproductive period of dairy heifers, which can be accomplished by breeding heifers earlier to reduce the age at first calving (Abeni et al., 2000). Many studies suggest that the optimal age at first calving is 24 months or younger (Mourits et al., 1999; Gabler and Heinrichs, 2003; Shamay et al., 2005; Stevenson et al., 2008). However, most of these researchers based their conclusions on milk production rather than whole economic measurements. Ettema and Santos (2004) found that only 2.7% of US Holstein dairy farms achieved the recommended targets of 24 months or younger at first calving with liveweight of at least 560 kg.

7. COSTS OF DELAYED CALVING

Following the costs of feeding the lactating herd, heifer rearing is the second largest expense in dairy operations. This expense accounts for approximately 15–20% of the total costs of producing milk (Gabler et al., 2000; Hutchison et al., 2017).

The direct benefits of reducing the age at first calving from 24 to 22 months in Holstein include saving $150 in heifer-rearing costs (estimated at ~$75 per month per heifer). Indirect benefits derived from this 2-month reduction is an increase in lifetime milk yield of 632 kg, but an increase of 1% in stillbirths. If the value per calf is $200 per animal and milk price is $0.37 per kilogram, the estimated indirect benefits and costs of these traits are −$2 and $236 for stillbirths and increased lifetime milk, respectively (Hutchison et al., 2017). An improvement in the estrus detection rate of 0.30–0.50 would increase profit by €53 per cow per year (Inchaisri et al., 2010).

A dairy herd is a complex system consisting of two interconnected parts: the milking herd and the replacement herd. Management decisions regarding replacement policy can have profound associations on the profitability of costs of the farm as a whole. Although it is often pursued as a management target, young age at first calving does not always lead to the most profitable outcome in dairy herd management. Local conditions on each farm remain important, and a heifer-rearing period that is more intensive (age at first calving ≈ 24.5 months) can lead to a decline in fertility (calving interval, days open, and services per conception), resulting in an increase in the depreciation of costs. Milk yield has been shown to have a significant effect on net profits for farms studied. The group with the highest milk yield also had the highest profitability without subsidies. The probability for successful return on investment improves when the herd management is based on quality heifer rearing. Nevertheless, an optimal replacement policy does not guarantee good dairy farm profitability, which is greatly influenced by the changing prices of inputs and outputs for agricultural markets (Krpálková et al., 2014). Furthermore, heifer-rearing policy can affect fertility in upcoming lactations, which is another factor farmers must keep in mind when rearing heifers. Therefore, optimization of the calving interval is of great economic importance for a dairy farm (Ruiz et al., 1989).

On Dutch farms, the total costs of rearing vary between $1,800 and $2,200 per heifer (Mohd Nor et al., 2012). Per heifer, reducing first calving age by a month reduces the costs of rearing by $51–116 (Gabler et al., 2000; Mohd Nor et al., 2012).

At the cow level, although a month lower relative first calving age is expected to reduce the total cost of rearing by, on average, $77 per heifer (Mohd Nor et al., 2012), it creates additional loss in revenues by reducing milk yield 90 kg for first lactation. At the herd level, lowering the first calving age will produce additional savings by reducing the number of replacement heifers needed (Mourits et al., 2000).

8. AIMS OF THE STUDY

The aims of this study were to determine whether decent fertility results can be achieved in replacement heifers using Estrotect pressure-sensitive heat detection aids compared with ordinary visual heat detection.

The hypothesis of this study was decent results can be achieved using pressure-sensitive heat detection aids compared with ordinary visual heat detection.

9. MATERIALS AND METHODS

9.1. Animals and grouping

The study was conducted between March 2019 and June 2020 in the replacement herd of one commercial dairy farm in Tartu county, Estonia. This study included 278 heifers (234 Holstein and 44 Estonian Red heifers). Animals that were next to be inseminated was grouped in one part of the youngstock barn, which had a possibility to lock them up with headlocks installed to the feeding table. There were 39 cubicles in this part of barn, which was the maximum number of animals we could have at a time. The group was re-organized monthly as there was constantly new animals aging. All animals were 12.5–15.0 months of age. All included animals were inseminated for the first time.

Approximately two-thirds (172 heifers, 142 from Holstein and 30 from Estonian Red breeds) of animals in the group were randomly selected to receive a disposable Estrotect heat detection device (Estrotect, Rockway Inc, Tennessee, United States). In the remaining animals, heat signs were detected visually (106 heifers, 92 from Holstein and 14 from Estonian Red breeds). The two groups were uneven. Some of the animals were already served before the devices were attached in the group. Devices were only fixed in animals that were not inseminated previously. Animals that were inseminated previously were included in the visual heat detection group.

9.4. Implementation of Estrotect

Heat detection devices were fixed to the sacrum of heifers to detect when the animal had been mounted. After all dirt and loose hair had been removed by brushing the sacral area, Estrotect devices were attached with aerosol glue. The aerosol glue was sprayed to the place where the device was to be attached 1 minute before the fixing of device. After fixing, the device was pressed down for 1–2 minutes. This ensured that the device attached to the hair thoroughly. When the animals mounted each other, the surface of the device was rubbed and an orange color was expelled. The more the animal was mounted, the more color was expelled. According to the manufacturer, if 80% of the device is colored, the animal is in heat. All animals that had lost their devices were excluded from the analysis. After insemination, the used devices were removed from the animals.

9.5. Visual heat detection

For the animals in which heat was detected visually, multiple signs were viewed. Standing heat, mounting activity, general activity, vocalization, chin resting, and mucus discharge were all used signs to conclude that the animal was in heat. Heat was detected at least two times per day for a 30-minute period. Detection was performed between 7:00–9:00 AM and 5:00–7:00 PM. All animals were palpated rectally before service. This was performed to obtain an overview of the uterus tone, expel possible mucus discharge, and screen out obvious abnormal pathologies to be sure that the animal qualified for reproduction.

9.6. Inseminations

All animals according to the heat signs were artificially inseminated by the same insemination technician using semen from several different pure breed sires. All animals were served to the uterine body (*corpus uteri*) with one 0.25 ml dose each.

9.7. Pregnancy check

After service, all animals were controlled with ultrasound from day 28 post insemination to ensure the insemination had been successful and resulted in a pregnancy or not. The ultrasound equipment used was Easi-Scan – D (BCF Technology Ltd, Scotland, UK). Pregnant animals were removed from the study group. The pregnancies were rechecked approximately 60–70 days after AI. Animals without a pregnancy were left in this group to wait for re-insemination.

10. STATISTICAL ANALYSIS

The effect of heat detection method (Estrotect *vs* visual heat detection) on heifers' pregnancy rate after first insemination was studied using a logistic model that considered the effects of breed and breed by heat detection method interaction. Initially, the random effect of the insemination bull was also considered, but as the latter was estimated to be zero from the model and thus did not affect the results, it was excluded from the final model. The results are presented with standard errors and are considered statistically significant at $p \leq 0.05$. Statistical analyses were performed with R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

11. RESULTS

The Estrotect group had a lower pregnancy rate after first insemination than the visual heat detection group in both breeds: 66.9 ± 4.0% *vs.* 71.7 ± 4.7% among Estonian Holstein heifers ($p = 0.436$), and 36.7 ± 8.8% *vs.* 71.4 ± 12.1% among Estonian Red heifers ($p = 0.037$), respectively (Figure 8). However, due to the relatively small difference among Estonian Holstein heifers and the small number of inseminated Estonian Red heifers, no statistically significant difference was found between heat detection methods overall ($p = 0.129$). Additionally, the interaction effect of breed and heat detection method was not statistically significant ($p = 0.105$). At the same time, the pregnancy rate of Estonian Red heifers was significantly lower than that of Estonian Holstein heifers ($p = 0.012$).

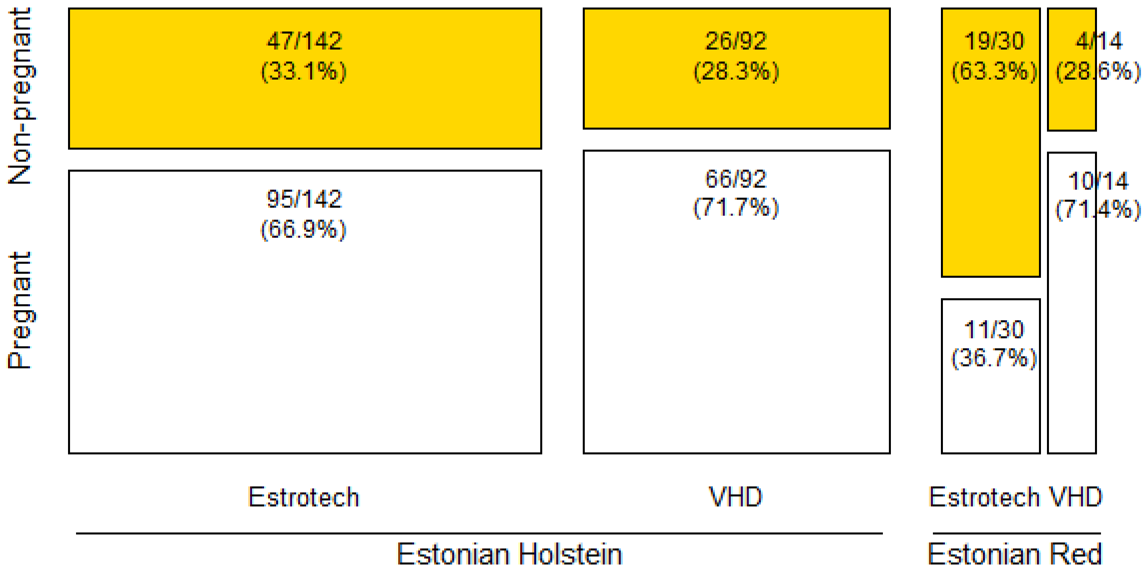

Figure 8. Distribution of the first insemination results (pregnant *vs.* nonpregnant) depending on breed and heat detection method (Estrotect *vs.* visual heat detection [VHD]).

12. LIMITATIONS OF THE STUDY

In this study, a relatively small number of inseminated animals were used. This study was conducted in only one farms replacement herd and, also precise visual detection achieved really good results.

13. DISCUSSION

Estrous expressions in dairy cows have been shortened and weakened. However, dairy heifers may not have such changes in estrous signs as those observed in cows because they have less stresses than cows**.** In this study, the results were in line with other studies (Stevenson et al., 2008., Patterson et al.,

2013.). The detection of estrus continues to be a problem on dairy farms. Undetected estrus or error in detection of estrus leads to increased days open and results in economic losses. Several factors affect the success of estrus detection in dairy replacement herds. For example, the time of day during which observations occur affects efficiency of detection, as well as the duration and frequency of the observations. These are all labor consuming, and especially farms that rely on visual heat detection benefit from well-managed heat detection programs. In this study, the high pregnancy rate (>71%) in the visual heat detection group indicates that the time spent on heat detection was sufficient, and the animals were correctly identified as expressing their heat before correctly timed insemination.
Maintaining a good first calving interval depends on good heat detection and correct timing of insemination. Heat detection should be made a high priority item in the herd. Someone should be made responsible for heat detection. In that person's absence, a backup person should be named and be made aware of the responsibilities. Time should be taken to educate everyone about the importance of this job, the signs to look for, when to look for them, and the benefits of a successful heat detection program.

Among the animals inseminated due to the Estrotect device, 66.9% of Holstein and 36.7% of Estonian Red breed heifers became pregnant. The difference between the breeds were interesting. There was not any specific reason for the difference in results. The amount of Estonian Red heifers was significantly smaller. Estonian Red heifers tended to be smaller and higher in body condition than Holstein heifers. Holstein heifers had a greater likelihood of resting their chin on the back of Estonian Red heifers and had better access to lick the device from their sacrum. Additionally, Estonian Red heifers tended to lay more often against the cubicle separators and walls. All these actions increased contact with the surface of the device and falsely expelled the color, making the device look activated. It might be that in this group had more false-positive animals, which were not in heat at all when inseminated. Holstein heifers in the same group reached much higher results with less exposure to these problems. Because 71.4% of Estonian Red heifers became pregnant from the first service by visual heat detection, it is unlikely that the difference is explained by the lower fertility of the Estonian Red heifers.
In this study, we had to use aerosol glue to stick the Estrotect devices to the animals so that they did not fall off when mounted. The instruction says that a precise cleaning of the sacral area followed by heating of the devices to body temperature before attaching ensures up to 8 weeks of functioning. By doing this as instructed, we had a high number of devices missing in the first 24 hours after attaching. After we started to use aerosol glue, only a few devices went missing during the study. Additionally, we discovered that when the new group of animals were gathered to the insemination pen, it was useless to immediately attach the devices. Animals were so excited that they rubbed the surface of

the device from each other. After that, we let the animals settle down to the new group for 2–7 days, and after this period, we attached the devices. Additionally, it was much more practical to lock the animals when they had learned to put their head through the headlock fence when fetching food.

Successful herd-level reproduction management is a complex job with many practical steps. People who practice it must be skillful to understand the estrous cycle and heat signs to trace the most optimal time to inseminate the animal in heat. Procedures in semen handling and thawing must be performed under certain guidelines to ensure the maximum amount of alive, forward-moving spermatozoa in the uterus. One of the most important factors is that the animal is in true estrus. The most common reason for unsuccessful service is that the animal has not been in proper estrus or the time of AI has failed. The crude AM-PM rule developed from earlier studies is still used today. Under this rule, when animals are found to show heat signs in the morning, they should be inseminated 12 hours later in the evening. However, new studies suggest that when the expressed heat signs are observed without information from additional devices or heat monitors, the animal should be serviced instantly. Insemination technique and the site of insemination might not affect the results for an individual animal, but in the large population, it has an effect to the conception rate. With transcervical, intrauterine AI, the highest conception rate can be achieved, compared with intracervical AI. Because the aim of whole fertility management is to maximize the conception rate and minimize the amount of services leading to pregnancy in the herd, all of these procedures should be performed carefully according to guidelines. Decreasing the age at first insemination for the heifers to 13 months lowers the age at first calving, which increases the herd's economical profit by cows entering the first lactation at a younger age. This practice might be performed if the growth rate of the heifers is at an efficient level, but primiparous cows tend to underperform in the first lactation. Finally, successful reproductive practices and increased conception rate are two key economic interests in modern-day dairy farming. In the case of problems with heat detection management, it is possible to use heat detection aids for improvement of replacement herd fertility.

14. CONCLUSION

According to this study, farms that are not satisfied with the reproduction performance in their replacement herd are struggling with labor problems or identification of animals in heat. Estrotect heat detection device could be helpful to increase the results in replacement herd fertility management. In particular, Holstein herds could benefit from this device. The grouping of the animals and attachment of the devices is time and labor consuming, but it can save time and labor requirements on a daily basis compared with those of proper visual heat detection management practices.

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